

# Simple Guidelines for Using the PHIDA tool

## What is PHIDA?

PHIDA is an excel workbook that includes two sheets with pre-defined settings and functions to analyze experimental data for comparing the host range of Bacteriophages Isolated Using Different Enrichment Methods. The first sheet enables researchers to configure the analysis and entering the experiment data. At the same time, the first sheet identifies outliers, OD growth trend of each sample, the detection time, and the designation of each sample. The second sheet generates a tabular report to show the maximum OD, the detection time, and the designation of all samples see Figure 1.

Serial	Samples	Avg <sub>last3</sub>	%OD <sub>max</sub>	D Time	Designation
1	Blank	0.1990	16.08%	0:00:00	0
2	Control	1.2374	100.00%	9:00:00	N
3	C-1	0.0000	0.00%	NA	C
4	D	0.6952	56.18%	10:00:00	D
5	NL	0.6952	56.18%	9:00:00	N/L+

Figure 1: the PHIDA report

The first sheet consists of three areas, as shown in Figure 2. The first area is the Parameter Area and starts from row 2 to row 12. This area allows researchers to enter different parameters to configure the analysis task. The second area is the Analysis Area, which starts from row 13 to row 19. This area identifies and displays a set of metrics, OD growth trends, and each sample's designation. The third area is the Data Area, allowing researchers to enter the experimental data for all samples.

Experiment:		suggested		actual		Blank Analysis		suggested actual			Control Analysis		1 suggested actual		
14	Analysis Area: from row 13 to row 19	Upper	0.199			AVG <sub>last3</sub>	0.199	Upper	2.176	AVG <sub>last3</sub>	1.23744444	Upper	1		
15		Lower	0.199			% OD <sub>max</sub> (Normalized)	16.08%	Lower	-4.394	% OD <sub>max</sub> (Normalized)	100.00%	Lower	0.199		
16		#outliers	21			D Time	0:00:00	#outliers	0	D Time	9:00:00	#outliers	27		
17						Designation				Detection Delay	0.00				
18		OD Growth Trends						OD Growth Trends				OD Growth Trends			
19		● 4.26251E-32	● 4.26E-32	● 4E-32				● 2.2209	● 2.0826	● 2.096		● 4E-32	● 4E-32	● 4E-32	
20	Time	Blank			Avg	avg-blank	Control			Avg	avg-blank	C-1			
21	0:0:0	0.199	0.199	0.199	0.199	0.000	0.198	0.196	0.211	0.202	0.003	0.199	0.199	0.199	
22	0:30:00	0.199	0.199	0.199	0.199	0.000	0.198	0.196	0.209	0.201	0.002	0.199	0.199	0.199	
23	Data area: from row 20 to the end	0.199	0.199	0.199	0.199	0.000	0.198	0.196	0.189	0.194	-0.005	0.199	0.199	0.199	
24		0.199	0.199	0.199	0.199	0.000	0.198	0.196	0.189	0.194	-0.005	0.199	0.199	0.199	
25		0.199	0.199	0.199	0.199	0.000	0.198	0.196	0.189	0.194	-0.005	0.199	0.199	0.199	
26	2:30:00	0.199	0.199	0.199	0.199	0.000	0.198	0.197	0.190	0.195	-0.004	0.199	0.199	0.199	
27	3:00:00	0.199	0.199	0.199	0.199	0.000	0.198	0.197	0.190	0.195	-0.004	0.199	0.199	0.199	
28	3:30:00	0.199	0.199	0.199	0.199	0.000	0.199	0.197	0.190	0.195	-0.004	0.199	0.199	0.199	

Figure 2: the PHIDA first sheet's areas

## How to use the PHDIA tool?

- 1) Complete experiment details
  - a. Title, date, user, MOI, etc...
  - b. Starting time (h, min, sec): the starting time of the experiment ( the time of the first reading)
  - c. Increments (minutes): how many minutes between two readings
  - d. Last row: the row number of the last line in the data. If your experiment includes one reading every 30 minutes for 24 hours, you should enter 61 in the last row to allow 48 entries starting from row 13 (the first row in the data area).
- 2) Copy results (in triplicates) to Analysis File
  - a. Blank-> Blank, Control-> Control, Samples->Samples
  - b. Use a new spreadsheet for each new host control - a single 394-well plate can accommodate four phages plus controls.
- 3) Review dataset and correct for outliers
  - a. Check growth trends agree between the triplicates
- 4) The **PHDIA** tool will automatically subtract blank values and calculate the difference between experiment value and control value to determine:
  - a. Detection delay (see Reference section for term definition)
  - b. %ODmax
- 5) The **PHDIA tool** will automatically assign lysis profile designation based on the values calculated above. See the Reference section for designations and their definitions.
- 6) The **PHDIA tool** will generate an automatic report

**Reference:**

Designation	Detection Delay (hr)	OD <sub>max</sub>	Definition and Criteria
C	$\Delta D = \text{N/A}$ Because sample would not have a D value	N/A	<b>Complete inhibition of the bacterial growth</b> <ul style="list-style-type: none"> <li>- sample never reach detection threshold for the whole duration of the experiment</li> </ul>
D+	$5 \text{ hours} < \Delta D < (\text{experimental duration} - D_{\text{control}})$	N/A	<b>More than 5 hours delay in bacterial growth compared to control</b> <ul style="list-style-type: none"> <li>- time difference to reach detection threshold between sample and control is <math>\geq 5</math> hrs and <math>&lt; \text{"experiment duration time} - \text{detection time of the control"}</math></li> </ul>
D	$1 < \Delta D < 5 \text{ hours}$	N/A	<b>Less than 5 hours delay in bacterial growth compared to control</b> <ul style="list-style-type: none"> <li>- time difference to reach detection threshold between sample and control is <math>\geq 1</math> and <math>&lt; 5</math> hrs)</li> </ul>
N	$\Delta D < 1 \text{ hour}$	$> 85\% \text{ control}$	<b>No effect of phage on bacterial growth</b> <ul style="list-style-type: none"> <li>- time difference to reach detection threshold between sample and control is <math>&lt; 1</math> hrs</li> <li>- OD<sub>max</sub> is <math>&gt; 85\%</math> of control</li> </ul>
N/L		$70 < \text{OD}_{\text{max}} \leq 85\%$	<b>Small effect on bacterial growth endpoint</b> <ul style="list-style-type: none"> <li>- time difference to reach detection threshold between sample and control is <math>&lt; 1</math> hrs</li> <li>- OD<sub>max</sub> is 70-85% of control's OD<sub>max</sub> <math>0.70 &lt; \% \text{OD}_{\text{max}} \leq 0.85</math></li> </ul>
N/L+		$40 < \text{OD}_{\text{max}} \leq 70\%$	<b>Moderate effect on bacterial growth endpoint</b> <ul style="list-style-type: none"> <li>- time difference to reach detection threshold between sample and control is <math>&lt; 1</math> hrs</li> <li>- OD<sub>max</sub> is 40-70% of control's OD<sub>max</sub> <math>0.40 &lt; \% \text{OD}_{\text{max}} \leq 0.70</math></li> </ul>
N/L++		$40\% \leq \text{OD}_{\text{max}}$	<b>Large effect on bacterial growth endpoint</b> <ul style="list-style-type: none"> <li>- time difference to reach detection threshold between sample and control is <math>&lt; 1</math> hrs</li> <li>- OD<sub>max</sub> is <math>\leq 40\%</math> of control</li> </ul>